UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

October 20, 2004

MEMORANDUM

Subject: Efficacy Review for *Selective Micro® Clean-Alpha*, EPA Reg. No. 74986-U; DP Barcode: D305847

From: Ibrahim Laniyan, Microbiologist
Product Science Branch
Antimicrobials Division (7510C)

Thru: Nancy Whyte, Acting Team Leader
Product Science Branch
Antimicrobials Division (7510C)

Michele E. Wingfield, Chief
Product Science Branch
Antimicrobials Division (7510C)

To: Emily Mitchell / Wanda Mitchell
Regulatory Management Branch II
Antimicrobials Division (7510C)

Applicant: Selective Micro Technologies, LLC
66 Cherry Hill Drive, Suite 230
Beverly, MA 01915

Formulation from the Label:

Active Ingredient(s)	% by wt.
Sodium chlorite	30.5 %
Inert Ingredients	69.5 %
Total	100.0 %

Amount of Chlorine Dioxide generated = 0.05%

I. BACKGROUND

The product, *Selective Micro® Clean-Alpha* (File Symbol 74986-U), is a new product. The applicant requested to register the product as a disinfectant (bactericide), sanitizer, tuberculocide, virucide, fungicide, algaecide, slimicide and deodorizer for use on hard, non-porous surfaces, including for use in medical and veterinary clinics, food and beverage processing facilities, food establishments, animal confinement facilities, and institutions. The product is not for use in households or where young children may be present. This product may be used to reduce microbial populations in potable water holding tanks and lines, in non-potable water systems in horticultural settings, on cut flowers, fruits and vegetable. The product can also be used for general disinfectant, sanitizer, algaecide and fungicide for horticultural and greenhouse applications. Dry sodium chlorite (considered the active ingredient) and [redacted] are packaged in separate pouches and placed together in another **2 liter-pouch** until use. Neither of the chemicals in the pouches have any pesticidal properties until combined together in water to produce chlorine dioxide, which does have antimicrobial activity. All studies were conducted at MicroBioTest, Inc., located at 105B Carpenter Drive in Sterling, VA 20164, and ATS Labs, located at 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121.

Previously submitted and reviewed studies, MRIDs 457782-08, 457782-09, 457782-11, and 458978-01, support the product's for the following uses against the organisms:

Food-contact sanitizer at 5ppm against *Staphylococcus aureus* and *Escherichia coli* (MRID# 457782-11).

Non-food-contact, non-porous hard surface sanitizer at 20ppm against *Staphylococcus aureus* and *Klebsiella pneumoniae* (MRID# 458978-01).

Disinfectant at 100ppm against *Staphylococcus aureus* (MRSA), *Enterococcus faecalis* (VRE), *Staphylococcus aureus*, *Salmonella choleraesuis*, and *Pseudomonas aeruginosa* (MRID# 457782-08 and MRID# 457782-09).

This data package contained correspondence from the applicant's representative (dated May 28, 2004), seven studies (MRID Nos. 462869-04 through 462269-10), Statements of No Data Confidentiality Claims for all seven studies, the proposed label, and Technical Bulletin 2004-01-Alpha.

Note: A number of the laboratory reports describe studies conducted for the product, 2L500. According to the laboratory report assigned MRID No. 462866-07, *Selective Micro® Clean-Alpha*, which is the subject of this efficacy report, is an alternate name for the product, 2L500 Chlorine Dioxide.

Note: The applicant's representative (in a letter to EPA dated May 28, 2004) states that the activated use solution for the product, *Selective Micro® Clean-Alpha*, is identical in all respects to the activated use solution for the registered product, *Selective Micro® Clean-A* (EPA Reg. No. 74986-1). Thus, efficacy data developed to substantiate claims for the product, *Selective Micro® Clean-A*, may also be used to substantiate claims for the product, *Selective Micro® Clean-Alpha*.

II. USE DIRECTIONS

The product is designed to be used for disinfecting hard, non-porous surfaces such as

tanks, transfer lines, food processing equipment, equipment, tile floors, walls, ceilings, stainless steel cold rooms, walk-in incubators, bench tops, biological hoods, incubators, and instruments. The product also is designed for sanitizing previously cleaned food preparation surfaces such as fountain drink and beverage dispensers; glassware, plates, and eating utensils; food processing equipment, including beer processing equipment and lines; and food conveyor belts. This product may be used to reduce microbial populations in potable water holding tanks and lines and in non-potable water used with cut flowers to minimize microbial transfer from water to flower. The product can also be used for general disinfectant, sanitizer, algaecide and fungicide in horticultural and greenhouse applications.

The proposed label provided the following instructions for activating the product: Fill the pouch with 2 liters of tap water. Wait at least 6 hours before use to ensure that the solution reaches full strength. Shake gently before use. Before use, verify the concentration using *Selective Micro® Chlorine Dioxide Test Strips*. Activate the product prior to the expiration date stamped on the pouch. Use the activated solution within 15 days of activation. Keep the activated solution in cool place out of direct sunlight.

The proposed label directions included the followings for the use of the product as a sanitizer, disinfectant, and general-purpose antimicrobial: For all applications, clean surfaces before using product. Apply by mop, sponge, or sprayer, ensuring visible wetness for times specified for these applications, or apply through immersion or clean-in-place application. Wear a NIOSH/MSHA-approved respirator appropriate for chlorine dioxide when using a high-pressure sprayer.

When sanitizing food contact surfaces, use a **5ppm solution**. For sanitizing non-food contact surfaces, use a **20ppm solution**. When disinfecting surfaces, use a **50ppm** or **100ppm solution**. See Technical Bulletin for specific directions for dilution and application.

Technical Bulletin 2004-01-Alpha repeated information on the proposed label and provided the following information:

To prepare a **500ppm solution** (one pouch in 2 liters or ½ gallon of water), and alternative dilution concentrations to target end-concentrations of chlorine dioxide inside closed Un-approved (or comparable to Un-approved) container (High Density Polyethylene, Polypropylene, Polyethylene Terephthalate, Polyvinyl Chloride, Polycarbonate, UV-blocking Glass, Gasket materials: silicone, viton or EPDM).

- For food-contact non-porous, hard surface sanitizing: Dilute 1:100 to obtain 5ppm solution and apply for 1 minute. Allow surfaces or equipment to air dry. Do not re-use solution. Do not rinse sanitized food contact surfaces.

- For non-food-contact non-porous, hard surface sanitizing: Dilute 1:25 to obtain 20ppm solution and apply for 5 minutes. Allow surfaces or equipment to air dry. Do not re-use solution. Do not rinse sanitized food contact surfaces.

- For hard, non-porous surface disinfection including medical and animal uses: Dilute 1:5 to obtain 100ppm solution and apply for 10 minutes. Allow surfaces or equipment to air dry. Do not re-use solution. Do not rinse sanitized food contact surfaces.

- For disinfection for clean-in-place applications for potable water systems: Dilute 1:5 to obtain 100ppm solution and apply for 10 minutes or longer. Drain tanks and lines. Rinse with potable water.

- For general antimicrobial applications for non-potable water systems in horticulture settings: Dilute 1:100 to obtain 5ppm solution.

- For reducing microbial populations in general cleaning and antimicrobial applications for water lines and tanks in potable water systems: Use 5ppm to 50ppm solution, apply overnight, and rinse with potable water.

- For horticultural disinfectant, sanitizer, algaecide, fungicide, and **biofilm** remover/inhibiter: Use, respectively, 100ppm solution for 10 minutes or more or 50ppm solution for 20 minutes or more, 5ppm for **1 minute**, and 50ppm for 20 minutes to overnight (algaecide, fungicide and **biofilm**).

- To extent shelf-life and freshness of fruits and vegetables in food processing facilities, dilute 1:100 to obtain 5ppm solution and apply for 1 minute. Allow surfaces or equipment to air dry. Do not re-use solution. Do not rinse sanitized food contact surfaces.

III. AGENCY STANDARD FOR PROPOSED CLAIMS

Confirmatory Efficacy Data Requirements: Under certain circumstances, an applicant is permitted to rely on previously submitted efficacy data to support an application or amendment for registration of a product and to submit only minimal confirmatory efficacy data on his own product to demonstrate his ability to produce an effective formation. This includes a minor formulation change (e.g., a change in an inert ingredient) in a registered product. Confirmatory data must be developed on the applicant's own finished product. For hospital disinfectants, 10 carriers on each of 2 samples representing 2 different batches of product must be tested against *Salmonella choleraesuis* (ATCC 10708), *Staphylococcus aureus* (ATCC 6538), and *Pseudomonas aeruginosa* (ATCC 15442) using either the AOAC Use-Dilution Method or the AOAC Germicidal Spray Products as Disinfectants Method. **Performance Standard:** Killing on all carriers is required. The above Agency standards are presented in DIS/TSS-5.

Sanitizing Rinses for Previously Cleaned Food Contact Surfaces – Additional Microorganisms: There are cases where an applicant requests to make claims of effectiveness against additional microorganisms for a product that is already registered as a sanitizing rinse for previously cleaned food contact surfaces. The Agency DIS/TSS guidance is silent on this matter. Confirmatory test standards would apply. For sanitizing rinses for previously cleaned food contact surfaces, 2 product samples, representing 2 different batches, must be tested against each additional microorganism. **Performance standard:** Acceptable results must demonstrate a 99.999% reduction in the number of microorganisms within 30 seconds. The results must be reported according to the actual count and percentage reduction over the control. Furthermore, according to information in the above AOAC test method itself, counts on the numbers control for the product should fall between 75 and 125 x 10^6/ml for percent reductions to be considered valid. The minimum concentration of the product which provides the results required above is the minimum effective concentration. Label directions for use, however, must state that a contact time

of at least 1 minute is required for sanitization. The above Agency standards are presented in DIS/TSS-4 and -17, as well as the AOAC Germicidal and Detergent Sanitizing Action of Disinfectants Method.

Tuberculocidal requirements: Disinfectants may bear additional label claims of effectiveness as tuberculocides when supported by appropriate tuberculocidal effectiveness data. Certain chemical classes (i.e., glutaraldehyde and quaternary ammonium compounds) are required to undergo validation testing in addition to basic testing. Products that are formulated with other chemical groups do not require validation testing. Products may be tested using one of four recommended methods: the AOAC Tuberculocidal Test Method, Tuberculocidal Activity of Disinfectants Test Method with significant modification of the standard test conditions of contact time and/or temperature, Quantitative Tuberculocidal Activity Test Method, and AOAC Germicidal Spray Products Test Method.

When using the existing or modified AOAC Tuberculocidal Activity Test Methods, or the AOAC Germicidal Spray Products Test Method, ten (10) carriers for each of two samples, representing two different batches of product, must be tested against *Mycobacterium bovis* BCG (a member of the *Mycobacterium tuberculosis* species complex). When using the existing or modified AOAC Tuberculocidal Activity Test Method, or the AOAC Germicidal Spray Products Test Method, killing on all carriers/slides as demonstrated in Modified Proskauer-Beck Broth, and no growth in any of the inoculated tubes of two additional media (i.e., Middlebrook 7H9 Broth Difco B, Kirchners Medium, and/or TB Broth Base) is required. Agency standards are presented in EPA DIS/TSS-6, Subdivision G Guidelines, and "EPA Data Call-in Notice for Tuberculocidal Claims," dated June 13, 1986.

Fungicidal requirements: Effectiveness of liquid disinfectants against specific pathogenic fungi must be supported by efficacy data derived from each of 2 samples representing 2 different batches using the AOAC Fungicidal Test. **Performance standard**. The highest dilution that kills all fungal spores is the minimum effective concentration.

Alternatively, the AOAC Use Dilution Method, modified to conform with appropriate elements in the AOAC Fungicidal Test, may be employed. If the product is intended for use as a spray, the AOAC Germicidal Spray Products Test must be employed. The inoculum in the above tests must be modified to provide a concentration of at least 10^6 conidia per carrier. Ten carriers on each of 2 samples representing 2 different batches must be employed in the test. **Performance requirements:** Killing of the test microorganism on all carriers is required. The above Agency standards are presented in DIS/TSS-06.

Note: As an interim policy, the Agency is accepting studies with dried carrier counts that are at least 10^4 for *Trichophyton mentagrophytes* and *Aspergillus niger*. The Agency recognizes laboratories are experiencing problems in maintaining dried carrier counts at the 10^6 level. This interim policy will be in effect until the Agency determines that the laboratories are able to achieve consistent carrier counts at the 10^6 level.

Virucidal requirements: The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of the AOAC Use-Dilution Method (for liquid disinfectants) must be used in developing data for virucides intended for use upon dry inanimate, environmental surfaces (e.g., floors, tables, cleaned dried medical instruments). To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use

on the product label. One surface for each of 2 different batches of disinfectant must be tested against a recoverable virus titer of at least 10^4 from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with the test results. **Performance standard:** For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level. These Agency standards are presented in DIS/TSS-7.

Supplemental Recommendations: An antimicrobial agent identified as a "one-step" cleaner-disinfectant, cleaner-sanitizer, or one intended to be effective in the presence of organic soil must be tested for efficacy with an appropriate organic soil load, such as 5% blood serum. The organic soil level suggested is considered appropriate for simulating lightly or moderately soiled surface conditions. When the surface to be treated has heavy soil deposits, a cleaning step must be recommended prior to application of the antimicrobial agent. The effectiveness of antimicrobial agents must be demonstrated in the presence of a specific organic soil at an appropriate concentration level when specifically claimed and/or indicated by the pattern of use. The hard water tolerance level may differ with the level of antimicrobial activity (e.g., sanitizer vs. disinfectant) claimed. To establish disinfectant efficacy in hard water, all microorganisms (i.e., bacteria, fungi, viruses) claimed to be controlled must be tested by the appropriate Recommended Method at the same hard water tolerance level. All products tested by the recommended methods may be tested at the exposure periods prescribed in those methods. When an antimicrobial agent is intended to be effective in treating a non-porous surface, the Recommended Methods simulate this condition by using non-porous surface carrier (stainless steel cylinder or glass slide) specified in the method. The exposure period or manner of use necessary to provide efficacy must be featured prominently on the product label. These Agency standards are presented in DIS/TSS-2.

IV. BRIEF DESCRIPTION OF THE DATA

1. MRID 462869-04 "AOAC Fungicidal Effectiveness Test" for Selective Micro® *Clean-Alpha*, by Felicia L. Sellers. Study conducted at MicroBioTest, Inc. Laboratory Project Identification Number 478-131. Study completion date – April 23, 2003.

This study was conducted against *Trichophyton mentagrophytes* (ATCC 9533). Two lots (Lot Nos. 1352-5 and 1353-10) of the product, 2L500 Chlorine Dioxide (*Selective Micro® Clean-Alpha*), were tested using AOAC Fungicidal Effectiveness Test (copy provided). One pouch of each lot of product was activated overnight (at least 15 hours) in two liters of 250±2.9% ppm AOAC hard water. A use solution was prepared by diluting the product using 250±2.9% ppm AOAC hard water to achieve 100ppm and 50ppm Chlorine dioxide. No serum was added to the inoculum. Neopeptone Glocose Broth (NGB) containing 0.1% $Na_2S_2O_3$ (NGB+) was used as the neutralizing subculture media. A 0.5 ml of test spore suspension was added to 10 replicates of 5 ml aliquot of each dilution of product and swirled. After respectively 10 and 20 minutes of exposure periods, a 4 mm loopful (10 µl) of test mixture was transferred into 10 ml of first subculture (NGB+), then to the second subculture (NGB), and both were scored after 10 days at 25-30°C for growth or no growth. Controls included viability control, initial counts, fungistasis control, sterility control, and confirmation of challenge fungus. The reported conidia-forming units (CFU) per ml in the initial inoculum was: ***Trichophyton mentagrophyte* 5.1 x 10^6**.

Note: The laboratory used a HACH tester and Chlorine Dioxide Strips, supplied by the applicant, to determine the chlorine dioxide concentration of the use solution.
Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

2. MRID 459139-03 "AOAC Use Dilution Test Supplemental" for Selective Micro® *Clean-Alpha*, by Felicia L. Sellers. Study conducted at MicroBioTest, Inc. Laboratory Project Identification Number 478-137. Study completion date – May 1, 2003.

This study was conducted against *Staphylococcus aureus*-MRSA (ATCC 33592) and *Enterococcus faecalis*-VRE (ATCC 51299) in the presence of a 5% organic soil load (heat-inactivated horse serum). Two lots (Lot Nos. 030503LMA and 030603LMA) of the product, 2L500 Chlorine Dioxide (*Selective Micro® Clean-Alpha*), were tested using the AOAC Use Dilution Test Supplemental (copy provided). One pouch of each lot of product was activated overnight (at least 15 hours) in two liters of 250±2.9% ppm AOAC hard water. A 50ppm use solution was prepared by diluting the product using 250±2.9% ppm AOAC hard water. Ten (10) stainless steel penicylinder carriers per lot and per organism were immersed in 20 ml of a 48-54 hour old suspension of the test organism for 15 minutes. The carriers were dried for 20-40 minutes at 37±2°C. Each carrier was exposed to the use solution for 20 minutes at 20±2°C. The neutralizer was Letheen Broth containing 0.1% sodium thiosulfate. Subcultures were incubated at 37±2°C for 48±2 hours. Controls included sterility, neutralizer effectiveness, dried carrier counts, viability, bacteriostasis, and confirmation of challenge microorganisms. The reported average colony forming units per carrier, for each test microorganism, are as follows: ***Staphylococcus aureus*-MRSA 1.3 x 10^5** and ***Enterococcus faecalis*-VRE 1.4 x 10^5**.

Note: The laboratory used a HACH tester and Chlorine Dioxide Strips, supplied by the applicant, to determine the chlorine dioxide concentration of the use solution.
Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

3. MRID 462869-06 "AOAC Use Dilution Test Confirmatory" for Selective Micro® *Clean-Alpha*, by Felicia L. Sellers. Study conducted at MicroBioTest, Inc. Laboratory Project Identification Number 478-138. Study completion date – May 8, 2003.

This study was conducted against *Pseudomonas aeruginosa* (ATCC 15442), *Staphylococcus aureus* (ATCC 6538), and *Salmonella choleraesuis* (ATCC 10708) in the presence of a 5% organic soil load (heat-inactivated horse serum). Two lots (Lot Nos. 030503LMA and 030603LMA) of the product, 2L500 Chlorine Dioxide (*Selective Micro® Clean-Alpha*), were tested using the AOAC Use Dilution Test Supplemental (copy provided). One pouch of each lot of product was activated overnight (at least 15 hours) in two liters of 250ppm AOAC hard water. A 50ppm use solution was prepared by diluting the product using 250ppm AOAC hard water. Ten (10) stainless steel penicylinder carriers per lot and per organism were immersed in 20 ml of a 48-54 hour old suspension of the test organism for 15 minutes. The carriers were dried for 20-40 minutes at 37±2°C. The carriers were exposed to the use solution for 20 minutes at 20±2°C. Each carrier was exposed to 10 ml of the use solution for 20 minutes at 20±2°C. Following exposure, each exposed carrier was then added to Letheen Broth containing 0.1% sodium thiosulfate. The carriers were incubated at 37±2°C for 48±2 hours, and examined for visible or no visible growth. Controls included dried carrier counts, viability, bacteriostasis, sterility, neutralizer effectiveness, and confirmation of challenge microorganisms. The reported average colony forming units per carrier, for each test microorganism, are as follows: ***Salmonella choleraesuis* 4.4 x 10^4**,

Staphylococcus aureus **1.4 x 10^5**, and ***Pseudomonas aeruginosa*** **1.5 x 10^5**.

Note: The laboratory used a HACH tester and Chlorine Dioxide Strips, supplied by the applicant, to determine the chlorine dioxide concentration of the use solution.
Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

4. MRID 462869-07 "AOAC Tuberculocidal Activity of Disinfectants" for Selective Micro® *Clean-Alpha*, by Angela L. Hollingsworth. Study conducted at MicroBioTest, Inc. Laboratory Project Identification Number 478-141. Study completion date – December 4, 2003.

This study was conducted against *Mycobacterium bovis*, BCG (Organon Teknika, Corp.). Two lots (Lot Nos. 030603LMA and 030703EUA) of the product, 2L500 Chlorine Dioxide (*Selective Micro® Clean-Alpha*), were tested using the AOAC Tuberculocidal Activity of Disinfectants Method (modified) as described in the AOAC Official Methods of Analysis, 16th Edition, 1995 (copy provided). One pouch of each lot of product was activated (at least 6 hours) in two liters of 250ppm AOAC hard water. The bag was allowed to sit overnight at room temperature until use. A 100ppm use solution was prepared by diluting the product using 250ppm AOAC hard water. Heat-inactivated horse serum was added to the inocula at a concentration of 5%, to simulate an organic soil load; Modified Proskauer-Beck Medium (MPBM) containing 0.1% $Na_2S_2O_3$ (MPBM+) was used as the neutralizing subculture media. Porcelain penicylinder carriers were immersed for 15 minutes in a 14-25 day old broth culture of the test organism. The carriers were dried for 30 minutes at 37±2°C. For each product lot, 10 contaminated carriers were transferred to individual tubes containing 10 ml of the product. The carriers were exposed to the product for 10 minutes at 20±2°C. Following exposure, each carrier was transferred to 10 ml of MPBM+ to neutralize. After at least 10 minutes, each carrier was transferred to a tube containing 20 ml of MPBM. From each tube of neutralizer, 2 ml aliquots were subcultured to individual tubes containing 20 ml of Middlebrook 7H9 Broth and 2 ml aliquots were subcultured to individual tubes containing 20 ml of Kirchner Medium. All tubes used for primary and secondary transfers were incubated for 60 days at 37±2°C and examined for the presence or absence of visible growth. All plates were incubated for 14-25 days at 37±2°C. When no growth was observed, culture tubes were incubated an additional 30 days. Controls included those for carrier counts, viability, neutralizer effectiveness, sterility, and confirmation of the challenge microorganism. The reported average Colony Forming Units (CFU) per carrier, for the test microorganism, is: ***Mycobacterium bovis*** **BCG 1.1 x 10^4**.

Note: The laboratory used a HACH tester and Chlorine Dioxide Strips, supplied by the applicant, to determine the chlorine dioxide concentration of the use solution.
Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

5. MRID 462869-08 "Germicidal and Detergent Sanitizing Action of Disinfectants" for Selective Micro® *Clean-Alpha*, by Angela L. Hollingsworth. Study conducted at MicroBioTest, Inc. Laboratory Project Identification Number 478-145. Study completion date – December 4, 2003.

This study was conducted against *Escherichia coli*, O157:H7 (ATCC 43895) and *Listeria monocytogenes* (ATCC 13932). Two lots (Lot Nos. 030503LMA and 030603LMA) of the product, *Selective Micro® Clean Alpha* (2L500), were tested using the AOAC Germicidal and Detergent Sanitizing Action of Disinfectants Method as described in the AOAC Official Methods of Analysis,

16th Edition, 1995 (copy provided). One pouch of each lot of product was activated overnight (at least 15 hours) in two liters of 250ppm AOAC hard water. A use solution of 5ppm chlorine dioxide was prepared by diluting the product using 250 ppm AOAC hard water to achieve 50ppm (equivalent to 27.5ppm reading on HACH) then 5ppm Chlorine dioxide (equivalent to 2.75ppm reading on HACH). Ninety-nine (99) ml of the use solution was added to a sterile, 250 ml Erlenmeyer flask at 20±2°C. One (1) ml bacterial suspension was added to the flask as the flask was swirled. After 30 and 60 second exposure periods, 1 ml of the bacterium-use solution mixture was transferred to phosphate buffered saline containing 0.1% sodium thiosulfate (PBW+) to neutralize. Neutralizer tubes were mixed well, serially diluted in PBS, and plated onto TGE agar plates. The plates were incubated for 2 days at 37±2°C and colonies were counted. Controls included numbers control, neutralizer effectiveness, sterility, and confirmation of challenge microorganisms. The reported average initial CFU/ml, for each test microorganism, are as follows: ***Escherichia coli*, O157:H7 8.1 x 10^7** and ***Listeria monocytogenes* 9.8 x 10^7.**

Note: The laboratory used a HACH tester and Chlorine Dioxide Strips, supplied by the applicant, to determine the chlorine dioxide concentration of the use solution.
Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

6. MRID 462869-09 "Germicidal and Detergent Sanitizing Action of Disinfectants" for Selective Micro® *Clean-Alpha*, by Angela L. Hollingsworth. Study conducted at MicroBioTest, Inc. Laboratory Project Identification Number 478-146. Study completion date – December 4, 2003.

This study was conducted against *Salmonella typhimurium*, (MDRS) CI 01005 (University of Maryland). Two lots (Lot Nos. 030503LMA and 030603LMA) of the product, *Selective Micro® Clean Alpha* (2L500), were tested using the AOAC Germicidal and Detergent Sanitizing Action of Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995 (copy provided). One pouch of each lot of product was activated overnight (at least 15 hours) in two liters of 250ppm AOAC hard water. A use solution of 5ppm chlorine dioxide was prepared by diluting the product using 250 ppm AOAC hard water to achieve 50ppm (equivalent to 27.5ppm reading on HACH) then 5ppm Chlorine dioxide (equivalent to 2.75ppm reading on HACH). Ninety-nine (99) ml of the use solution was added to a sterile, 250 ml Erlenmeyer flask at 20±2°C. One (1) ml bacterial suspension was added to the flask as the flask was swirled. After 30 and 60 second exposure periods, 1 ml of the bacterium-use solution mixture was transferred to phosphate buffered saline containing 0.1% sodium thiosulfate (PBW+) to neutralize. Neutralizer tubes were mixed well, serially diluted in PBS, and plated onto TGE agar plates. The plates were incubated for 2 days at 37±2°C and colonies were counted. Controls included numbers control, neutralizer effectiveness, sterility, and confirmation of challenge microorganisms. The reported average initial CFU/ml, for the test microorganism, is: ***Salmonella typhimurium*, (MDRS) 8.4 x 10^7.**

Note: The laboratory used a HACH tester and Chlorine Dioxide Strips, supplied by the applicant, to determine the chlorine dioxide concentration of the use solution.
Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

7. MRID 462869-10 "Virucidal Efficacity of a Disinfectant for Use on Inanimate Environmental Surfaces" for Selective Micro® *Clean-Alpha*, by Angela L. Hollingsworth. Study conducted at ATS Labs. Laboratory Project Identification

Number A01696. Study completion date – January 27, 2004.

This study was conducted against Human Coronavirus (Strain 229E; ATCC VR-740), using MRC-5 cells (human embryonic lung cells; obtained from ViroMed Laboratories, Inc.) as the host system. Two lots (Lot Nos. 3302-01 and 3304-01) of the product, *Selective Micro® Clean Alpha* (2L500), were tested according to ATS Labs Protocol No. SMT01080603.COR (copy not provided). One pouch of each lot of product was activated overnight (at least 15 hours) in two liters of 250ppm AOAC hard water. A use solution of 100ppm chlorine dioxide was prepared by diluting the product using 250 ppm AOAC hard water to achieve 100ppm (equivalent to 32.1ppm reading on HACH). The stock virus culture contained a 5% organic soil load (fetal bovine serum). Films of virus were prepared by spreading 0.2 ml of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were dried at 20.1°C in a relative humidity of 48% for 20 minutes. For each lot of product, separate dried virus films were exposed to 2.0ml of the use dilution for 10 minutes. After exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixture was passed through a Sephadex column, and diluted serially in Eagle's minimal essential medium supplemented with 2% heat-inactivated fetal bovine serum, 10 µg/ml gentamicin, 100 units/ml penicillin, and 2.5 µg/ml amphotericin B. MRC-5 cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 ml of the dilutions. The cultures were incubated at 31-35°C in a humidified atmosphere of 5-7% CO_2 and scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for cytotoxicity, dried virus controls, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber. The titer of the dried virus control was **4.5 $\log_{10}$**. Taking the cytotoxicity and neutralization control results into consideration, the reduction in viral titer was $\geq$**4.0 $\log_{10}$** for both batches.

Note: The applicant provided the data for one failed trial. In that trial, an approximate 10ppm concentration of the test substance was inadvertently utilized in testing instead of the requested 100ppm. Thus, the data were invalid. These data were not used to evaluate efficacy of the test product. See Attachments I of the laboratory report.
Note: The laboratory used a HACH tester and Chlorine Dioxide Strips, supplied by the applicant, to determine the chlorine dioxide concentration of the use solution.
Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

V. RESULTS

MRID # 462869-10	Results			Dried Virus Control ($TCID_{50}$/0.1 ml)
		Lot 3302-01	Lot 3304-01	
Human Coronavirus	10^{-1} to 10^{-6} dilutions	Complete inactivation	Complete inactivation	$10^{4.5}$
	$TCID_{50}$/0.1 ml	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
	TCD_{50}/0.1 ml	$\leq 10^{0.5}$	$\leq 10^{0.5}$	

MRID # 462869-04	Lot #	Dilution Time	Media Type (No. Growth/No. Tested)		Average Initial CFU/ml
			NGB+	NGB	
Trichophyton mentagrophytes	1352-5	100ppm 10minutes	0/10	0/10	5.1×10^8
		50ppm 20minutes	0/10	0/10	
	1353-10	100ppm 10minutes	0/10	0/10	
		50ppm 20minutes	0/10	0/10	

MRID Number	Organism	No. Exhibiting Growth/Total No. Tested		Dried Carrier Counts(average CFU/carrier)
		Lot No. 030503LMA	Lot No. 030603LMA	
462869-06	*Staphylococcus aureus*	0/10	0/10	1.4×10^5
	Pseudomonas aeruginosa	0/10	0/10	1.5×10^5
	Salmonella choleraesuis	0/10	1/10	4.4×10^4
462869-05	*Staphylococcus aureus*- MRSA	0/10	0/10	1.3×10^5
	Enterococcus faecalis- VRE	0/10	0/10	1.4×10^5

MRID # 462869-07	Lot #	Media Type (No. Growth/No. Tested)				Average CFU/carrier
		MPBM+	MPBM	7H9	KM	
Mycobacterium bovis, BCG	030603LMA	0/10	0/10	0/10	0/10	1.1×10^4
	030703EUA	0/10	0/10	0/10	0/10	

MRID Number	Organism	Exposure Time	% Reduction		Numbers Control(average CFU/ml)
			Lot No. 030503LMA	Lot No. 030603LMA	
462869-08	*Escherichia coli*, O157:H7	30 sec.	>99.999	>99.999	8.1×10^7
		60 sec.	>99.999	>99.999	
	Listeria monocytogenes	30 sec.	<99.969	<99.969	9.8×10^7
		60 sec.	<99.969	<99.969	
462869-09	*Salmonella typhimurium*, (MDRS)	30 sec.	>99.999	>99.999	8.4×10^7
		60 sec.	>99.999	>99.999	

VI. CONCLUSIONS

1. The submitted efficacy data (MRID 462869-04) **support** the use of the product, 2L500 Chlorine Dioxide (*Selective Micro® Clean-Alpha*), as a disinfectant with fungicidal activity when tested against ***Trichophyton mentagrophyte***, at **100ppm** and **50ppm** for respectively **10 minutes** and **20 minutes** at 25±2°C in the presence of **250±2.9% ppm hard water**.

2. The submitted efficacy data (MRID 462869-05) **support** the use of the product, 2L500 Chlorine Dioxide (*Selective Micro® Clean-Alpha*), as a disinfectant with bactericidal activity when tested against ***Staphylococcus aureus*-MRSA** and ***Enterococcus faecalis*-VRE**, at **50ppm chlorine dioxide** and in the presence of **250±2.9% ppm hard water** and **5% organic soil load** (heat - inactivated horse serum) on hard non porous surfaces for a contact time of **20 minutes** at 20±2°C.

3. The submitted efficacy data (MRID 462869-06) **support** the use of the product, 2L500 Chlorine Dioxide (*Selective Micro® Clean-Alpha*), as a hospital disinfectant when tested against ***Salmonella choleraesuis***, ***Staphylococcus aureus***, and ***Pseudomonas aeruginosa***, at **50ppm chlorine dioxide** and in the presence of **250±2.9% ppm hard water** and **5% organic soil load** (heat - inactivated horse serum) on hard non porous surfaces for a contact time of **20 minutes** at 20±2°C.

4. The submitted efficacy data (MRID 462869-07) **support** the use of the product, 2L500 Chlorine Dioxide (*Selective Micro® Clean-Alpha*), as a disinfectant with bactericidal activity when tested against ***Mycobacterium bovis*, BCG**, at **100ppm chlorine dioxide** and in the presence of **250±2.9% ppm hard water** and **5% organic soil load** (heat -inactivated horse serum) on hard non porous surfaces for a contact time of **10 minutes** at 20±2°C.

5. The submitted efficacy data (MRID 462869-08) **support** the use of the product, ***Selective Micro® Clean Alpha* (2L500),** as a sanitizing rinse on previously cleaned, hard, non-porous, food contact surfaces against ***Escherichia coli*, O157:H7** in the presence of 250ppm hard water for a contact time of **30 seconds** and **1 minute** at **5ppm chlorine dioxide** at 25±2°C.

Efficacy data submitted in MRID No. 462869-08 **do not support** use of the product, ***Selective Micro® Clean Alpha* (2L500),**, as a sanitizing rinse on previously cleaned, hard, non-

porous, food contact surfaces against ***Listeria monocytogenes*** in the presence of 250ppm hard water for a contact time of **30 seconds** and **1 minute** at **5ppm chlorine dioxide** at 25±2°C. The proposed label makes no claim of efficacy against this organism.

6. The submitted efficacy data (MRID 462869-09) **support** the use of the product, *Selective Micro® Clean Alpha* (2L500), as a sanitizing rinse on previously cleaned, hard, non-porous, food contact surfaces against ***Salmonella typhimurium*, (MDRS)** in the presence of 250ppm hard water for a contact time of **30 seconds** and **1 minute** at **5ppm chlorine dioxide** at 25±2°C.

7. The submitted efficacy data (MRID 462869-10) **support** the use of the product, *Selective Micro® Clean Alpha* (2L500), as a disinfectant with virucidal activity on hard, non-porous surfaces when tested against **Human Coronavirus**, in the presence of 250ppm hard water for a contact time of **10 minutes** at **100ppm chlorine dioxide** at 20.1°C.
Please Note: Acceptance of this data to support a label claim for Human coronavirus (or canine coronavirus) does not support a label claim for this product as an effective disinfectant against the causative agent of Severe Acute Respiratory Syndrome (SARS).

VII. RECOMMENDATIONS

1. The terms "biofilm" and "slime" are consider, by the Agency, to be used, respectively, on consumer product labeling (public health organisms) and products that are used exclusively in the industrial/commercial setting (non-public health organisms). **The applicant must replace all biofilm claims with slime claims**.

2. The proposed label claims that the product, *Selective Micro® Clean-Alpha*, is effective as a fungicide against *Penicillium digitatum, Botrytis Sp.*, and *Fusarium solani*, at 5ppm for **one (1) minute**, **are not supported** by the applicant data ("AOAC Fungicidal Effectiveness Test" for Selective Micro® *Clean-Alpha*, by Felicia L. Sellers. Study conducted at MicroBioTest, Inc. Laboratory Project Identification Number 478-132. Study completion date – July 29, 2003) due to contact differences. **The data demonstrated effectiveness in 1 hour contact time. The applicant must change the contact time from 1 minute to one (1) hour to retain the fungicidal claims on non-public health organisms**.

3. The proposed label claims that the product, *Selective Micro® Clean-Alpha*, is an effective sanitizer rinse at 5ppm, in a solution of 250ppm AOAC hard, on previously cleaned food contact surfaces against ***Escherichia coli***, ***Staphylococcus aureus***, ***Escherichia coli* O157:H7**, and ***Salmonella typhimurium* (MDRS)** for a contact time of 1 minute at 20±2°C, **are supported** by the applicant's data.

4. The proposed label claims that the product, *Selective Micro® Clean-Alpha*, is an effective sanitizer rinse at 20ppm, in the presence of a 5% organic soil load, in a solution of 250ppm AOAC hard, on previously cleaned non-food-contact surfaces against ***Klebsiella pneumoniae*** and ***Staphylococcus aureus*** for a contact time of 1 minute at 20±2°C, **are supported** by the applicant's data.

5. The proposed label claims, as supported by applicant's data, **are acceptable** regarding the use of the product, *Selective Micro® Clean-Alpha*, as a disinfectant with **bactericidal** and

tuberculocidal activities at **100ppm** on hard, non-porous surfaces in the presence of a **5% organic soil load**, in a solution of **250ppm AOAC hard water**, against the following microorganisms for a contact time of **10 minutes** at room temperature (20±2°C):

Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella choleraesuis, **methicillin-resistant *S. aureus,*** **vancomycin-resistant *Enterococcus faecalis*, *Trichophyton mentagrophyte*, and *Mycobacterium bovis*.**

6. The proposed label claims, as supported by applicant's data, **are acceptable** regarding the use of the product, *Selective Micro® Clean-Alpha*, as a disinfectant with **bactericidal** activity at **100ppm** and **50ppm chlorine dioxide** on hard, non-porous surfaces in the presence of a **5% organic soil load**, in a solution of **250ppm AOAC hard water**, against the following microorganisms for a contact time of **10 minutes** and **20 minutes** respectively at room temperature (20±2°C):

***Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella choleraesuis*,** **methicillin-resistant *S. aureus*, and vancomycin-resistant *Enterococcus faecalis*.**

7. The applicant should make the following changes to the proposed label, as appropriate:

- On pages 1 and 2 of the proposed label, change "***E coli* 0157:H7, *Salmonella* (MDRS)**", under food -contact sanitizer claims, to read "***E coli* O157:H7, *Salmonella typhimurium* (MDRS)**".

- On pages 2 of the proposed label, change "***Tuberculosis* and *T-mentag.***", under disinfectant claims, to read "***Mycobacterium bovis* and *Trichophyton mentagrophyte***".

- In the "Directions for Use" section of the proposed label [see page 2 of the label], change all "**2000:1 dilution, 100:1 dilution, 25:1 dilution, 10:1 dilution, and 5:1 dilution**" to read "**1:2000 dilution, 1:100 dilution, 1:25 dilution, 1:10 dilution, and 1:5 dilution**".